STUDY DESIGN FOR THE TOXICITY EVALUATION OF AMMONIUM PERCHLORATE

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ABSTRACT

Ammonium perchlorate (AP), CAS registry number 7790-98-9, is used as a class 1.1 oxidizer in solid rocket engine propellant mixtures. Production and storage has resulted in the contamination of soil and water resources on government and contractor installations. In 1992 the US Environmental Protection Agency (EPA) established a provisional reference dose (RfD) of 1E-04 mg/kg/day ammonium perchlorate. Remediation levels will be based on this provisional RfD. While EPA was thorough in its evaluation of the literature cited, the analysis relied upon standard default uncertainty factors to determine the RfD. In light of recently available data, the provisional RfD may be overly conservative. If adopted, the provisional RfD will result in millions of dollars being spent on unnecessary cleanup.

INTRODUCTION

The use of ammonium perchlorate has led to soil and water contamination in some areas of the country. Remediation levels based on a provisional RfD proposed by the U.S. EPA will be overly conservative since their are insufficient toxicity data. This study design is part of a DOD effort to develop a toxicity database which can be used to establish a realistic remediation level. The toxicity study was designed to evaluate the potential of ammonium perchlorate to produce alterations in thyroid function by providing specific dose-response information and an initial estimate of the threshold amount of ammonium perchlorate necessary to produce a change in normal thyroid hormone levels. A two-stage, ninety day toxicity study will provide information for determining safe levels of exposure to ammonium perchlorate in drinking water.

The EPA has indicated a willingness to reevaluate the provisional RfD after new toxicity studies have been performed. Accordingly, the proposed study has been designed to evaluate the potential of AP to produce reproductive and developmental toxicity and produce alterations in thyroid function. A model of AP-induced thyroid effects will be developed and predictions using the rat-data derived model made for consequences of human exposures. This study will fill the existing data gaps for specific AP dose-response information, reproductive and developmental toxicity effects, and the threshold level for thyroid hormone effects. The animals will receive the test compound in drinking water as this route of treatment provides a more uniform dose than a single bolus dose produced by gavage and simulates the most probable route of human exposure in cases of environmental contamination.

STUDY DESIGN

PURPOSE OF THE STUDY

This study will evaluate the potential of AP to produce alterations in thyroid function and to determine the threshold dose for AP caused effects on thyroid hormone levels in rats. A model of AP-induced thyroid effects will be developed, and this model will form the basis for follow-on work using the Rhesus monkey to validate the predictions made with the rat-data derived model to human exposures. This study will also evaluate the potential of ammonium perchlorate (AP) to produce alterations in paternal fertility, maternal pregnancy and lactation, growth and development of offspring of Sprague-Dawley rats. Behavioral testing will be performed to determine any signs of development of neurotoxicity. The animals will receive the test compound in drinking water as this route of treatment provides a more uniform dose than a single bolus dose produced by gavage and simulates the most probable route of human exposure in cases of environmental contamination.

The proposed study will be performed following the OECD Screening Information Data Set (SIDS) protocol, as modified. Blood thyroglobulin (Tg), T_3, T_4, reverse T_3, and TSH will be determined. Thyroids will be weighed and volume will be measured at necropsy. Thyroid cellular architecture will be examined via morphometric procedures following the dosing period.

DOD RELEVANCY

This study is part of a proactive effort by the DOD to develop a toxicity database for AP which can be used to establish a scientifically based RfD. In the absence of relevant AP toxicity information, the provisional RfD proposed by EPA in December 1992 will become the de facto cleanup standard and will result in millions of DOD dollars being spent on unnecessary cleanup and restoration.

SCIENTIFIC OBJECTIVE

This study will evaluate the potential of AP to produce alterations in thyroid function and to determine the threshold dose for AP caused effects on thyroid hormone levels in rats. A model of AP-induced thyroid effects will be developed, and this model will form the basis for follow-on work using the Rhesus monkey to validate the predictions made with the rat-data derived model to human exposures. This study will also evaluate the potential of AP to produce alterations in paternal fertility, maternal pregnancy and lactation, growth and development of offspring of Sprague-Dawley rats.

TECHNICAL BACKGROUND

Human Data: The EPA report (ECAO, 1992) cites many studies concerning perchlorate toxicity. The sole epidemiologic study cited (Rockette and Arena, 1983) in the ECAO report was considered equivocal, in that the workers sampled in the study were exposed to many other chemicals in addition to perchlorate. Similarly, a search of the literature revealed only one human study of use in establishing a no adverse effect level (NOAEL) for AP. It was a clinical investigation (Stanbury and Wyngaarden, 1952) which involved potassium perchlorate in the treatment of Graves' disease. Unfortunately, that study was a very short term test in which the effects of perchlorate administration were measured over a six-hour period. Furthermore, Graves' disease involves the thyroid, which is a target organ for perchlorate toxicity.

Very limited pharmacokinetic data were found; one study conducted in two human subjects administered 784 g of sodium perchlorate per 100 g of water showed that 50% was eliminated in urine 5-hours after administration and 95% was eliminated 48-hours after administration (Durand, 1938).

Animal Data: An acute single dose, oral LD_{50} of 4200 mg/kg in "white rats" was reported by Shigan (1963, translated from the Russian, 1994). An unspecified number of "white rats" were administered AP by an unspecified route (probably gavage) under the following conditions: 650 mg/kg/day for 1 month, where Shigan concluded that AP "does not have noticeable cumulative properties", and 190 mg/kg/day for 3 months, where Shigan concluded that AP "at the test dose affects the regulation of the vegetative nervous system, causing a certain cholinergic activity.' ... "Furthermore, ammonium perchlorate causes a statistically reliable change in the protein fractions of the blood serum, a decrease in detoxification and the disruption of the glycogen-forming function of the liver". No other repeated exposure toxicity information is available for AP. Mannisto et al (1979) conducted limited work on thyroid effects in Sprague-Dawley rats with a related compound, potassium perchlorate, which is discussed below.

MECHANISM OF TOXICITY

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis and secretion of thyroid hormones. Perchlorate is known to competitively inhibit one of these steps, the iodine trapping mechanism, which incorporates free iodine into T_3 and T_4 (Capen, 1992). Low T_3 causes release of thyroid stimulating hormone (TSH) from the anterior pituitary, which results in thyroid follicular cell stimulation and hyperplasia. This hyperplasia may cause an increase in thyroid gland size (hypertrophy)

(ORD, 1988). The following physiological responses are expected to occur in an animal following perchlorate exposure:

1) Perchlorate will block the incorporation of free iodine into tri- and tetraiodothyronines (T_3 and T_4) by the thyroid gland.

2) In acute perchlorate exposures, this may be detected as either reduced radioiodine uptake by the thyroid or release of radioiodine already incorporated into the thyroid.

3) Chronic thyrotoxic effects of perchlorate may be detected as a drop in T_3/T_4 levels accompanied by an elevation in thyroid stimulating hormone (TSH) (Capen, 1992).

4) The thyroid gland should enlarge due to follicular cell hyperplasia subsequent to increased TSH levels (Brabant et al., 1992; Capen, personal communication, 1994).

5) Proliferation of thyrocytes (a hyperplastic response) may also progress to tumor formation (ORD, 1988, Hill, 1989).

Although the ECAO report does not derive a carcinogenic potency factor for perchlorate, it speculates that perchlorate is a class B2 "probable human" carcinogen. The report suggests that given the threshold mechanism for perchlorate goiterogenesis, the RfD should also be protective for potential carcinogenesis by perchlorate. This would appear valid, based on the following:

1) Depression of T_3/T_4 and elevation of TSH are cardinal signs of a disturbance of the thyroid-pituitary axis (Capen, 1992).

2) Experimental thyroid carcinogenesis resulting from prolonged administration of perchlorate is preceded by goiterogenesis, and hence, is thought to be mediated by the same iodine-blocking mechanism(s) described above.

3) Animals treated with perchlorate at carcinogenic levels are protected from thyroid carcinogenesis if given exogenous T_3/T_4 (Paytner et al, 1988).

If the thyroid-pituitary axis is not disturbed, there is no carcinogenic risk. Hence, the threshold concentration of perchlorate, i.e., the perchlorate concentration below which there is no depression of T_3/T_4 accompanied by TSH elevation, is completely protective against carcinogenesis. The EPA has endorsed this concept for thyroid follicular carcinogenesis which depends upon goitrogenesis resulting from derangement of the thyroid-pituitary axis, i.e., depressed T_3/T_4 with elevated TSH (Hill et al, 1989; Hill, personal communication, 1994). For these reasons, despite EPA's classification of perchlorate as a B2 carcinogen, there is no carcinogenic risk at levels below the threshold at which perchlorate disrupts the thyroid-pituitary axis.

EXPERIMENTAL DESIGN

This study is intended to fill the data gaps for specific dose-response information, reproductive and developmental toxicity effects, and the threshold level for thyroid hormone effects of AP. This study will determine the threshold dose of AP that causes effects on thyroid hormone levels and evaluate the potential of AP to produce alterations in paternal fertility, maternal pregnancy and lactation, and growth and development of offspring of Sprague-Dawley rats. The animals will receive the test compound in drinking water as this route of treatment provides a more uniform dose than a single bolus dose produced by gavage and simulates the most probable route of human exposure in cases of environmental contamination. The proposed study will be performed following the OEDC Screening Information Data Set (SIDS) protocol, as modified by the author.

A model of AP-induced thyroid effects will be developed, and this model will form the basis for follow-on work using the Rhesus monkey to validate the predictions made with the rat-data derived model for extrapolation to humans. This study will also evaluate the potential of ammonium perchlorate (AP) to produce alterations in paternal fertility, maternal pregnancy and lactation, growth and development of offspring of Sprague-Dawley rats. Behavioral testing will be performed to determine any signs of development of neurotoxicity. Blood thyroglobulin (Tg), T_3, T_4, reverse T_3, and TSH will be determined. Thyroids will be weighed and thyroid volume will be measured at necropsy. Thyroid cellular architecture will be examined via morphometric procedures following necropsy.

TWO-STAGE STUDY DESIGN

This study will employ a novel two-stage threshold design based on current EPA funded research conducted at Virginia Commonwealth University on optimal threshold designs (Schwartz et al, 1995). A two-stage study design with 3 doses (in addition to the control) in the first stage and 3 doses (in addition to control) in the second stage will maximize the threshold estimate. The first stage is used to determine the range of activity of the compound, AP effects on thyroid function in this instance, and the second stage is used to "patch" the deficiencies in the first stage. These deficiencies are caused by the incomplete knowledge of the dose-response relationship when the initial dose-response relationship is hypothesized. The two-stage procedure assumes that the experimental conditions in the two stages are equivalent and assigns animals to the dose groups in a ratio of approximately 1:2 for first stage and second stage studies (Myers et al).

The unit of comparison will be the individual male or female rat (or the litter). Results of the quantitative continuous variables (e.g. parental and pup body weights) will be intercompared for the treatment groups and control group by the use of Levene's test of equal variances, and analysis of variance (ANOVA). When Levene's test indicates homogeneous variances and the ANOVA is significant, a Bonferroni t-test will be used for pairwise comparisons. When Levene's test indicates heterogeneous variances, all groups will be compared by an ANOVA for unequal variances or an appropriate transformation will be done. Nonparametric data will be statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U test for pairwise comparisons when appropriate. Frequency data (such as the various indices) will be compared using the Fisher's Exact Test. For all statistical tests, the fiducial limit of 0.05 (two-tailed) will be used as the criterion for significance.

To derive an optimal two-stage quantal response threshold design for the perchlorate experiments, initial information about the dose-response relationship was estimated, as follows, from the findings of Mannisto et al (1979) and provided to Virginia Commonwealth University (Schwartz, personal communication):

- background rate of 0
- ED_{50} of 30 mg/kg/day
- threshold between 3 mg/kg/day and 10 mg/kg/day

The T-optimality criterion will result in optimal dose levels of perchlorate that minimize the variability associated with the threshold estimate. Schwartz (personal communication) suggested the following T-optimal doses of perchlorate for the first stage of the study based on my initial estimates (above):

- a control dose
- a very low dose, in the range of 0.1 mg/kg/day
- two higher dose groups, 27 mg/kg/day and 34 mg/kg/day

Based on the proportional responses at the first stage doses, the estimated threshold curve can be refined. The second stage optimal doses are determined using this estimated curve as the second stage initial information. The experiment is then conducted at the second stage optimal doses. Based on the proportional responses from the first stage doses and from the second stage optimal doses, the final threshold dose-response curve can be estimated. To obtain a good estimate of the slope of the dose-response curve, it is important that the two higher dose groups hit the region of activity on the dose-

response curve. However, since an estimated ED_{50} of 30 mg/kg/day results in a rather steep dose-response curve making it more difficult to determine the region of activity, a 14-day pilot study will be conducted to refine the ED_{50} estimate prior to conducting the two stage study.

PILOT STUDY

The 14-day pilot study will be conducted with eight groups of 12 rats (6 male and 6 female/group) for a total of 96 animals (50 of each sex will be ordered). Group assignment and dose levels will be as shown in Table 1. AP solution concentrations will be calculated using an estimated water consumption of 100 mL/day/rat and a threshold dose (ThD) of 10 mg/kg/day.

Table 1. Group Assignments and Dose Levels for Pilot Study

Group	No. of Animals Males	Females	Conc. (mg/L)	AP Target Dose (mg/kg body wt/day)
Control	6	6	0.0	0.0
Very Low	6	6	1.25	0.1
Low	6	6	5.0	1.0
Med.Low	6	6	12.5	5
Medium	6	6	25	10
Med.High	6	6	50	20
High	6	6	125	50
Very High	6	6	250	100

TWO STAGE STUDY

First Stage: There will be 72 animals (24 males and 48 females) assigned to the first stage at the initiation of the treatment period. A satellite group consisting of non-mated females will be used to determine if there are any differences in thyroid hormone levels due to gestational status. Group assignments and dose levels will be as shown in Table 2. Target dose and concentration to be determined from results of the 14-day study. Estimated water consumption to be used for calculation will be derived from 14-day study values.

Table 2. Group Assignments and Dose Levels for First Stage

Group	No. of Animals Males	Females (mated)	Females (satellite)	Conc. (mg/L)	AP Target Dose (mg/kg body wt/day)
Control	6	6	6	0.0	0.0
Low	6	6	6	TBD	TBD
Middle	6	6	6	TBD	TBD
High	6	6	6	TBD	TBD

Second Stage: There will be 144 animals (48 males and 96 females) assigned to the second stage at the initiation of the treatment period. A satellite group consisting of non-mated females will be used to determine if there are any differences in thyroid hormone levels due to gestational status.

Group assignments and dose levels will be as shown in Table 3. Target dose, concentration, and estimated water consumption to be based on the first stage study.

Table 3. Group Assignments and Dose Levels for Second Stage

Group	No. of Animals Males	Females (mated)	Females (satellite)	Conc. (mg/L)	Target Dose (mg/kg body wt/day)
Control	12	12	12	0.0	0.0
Low	12	12	12	TBD	TBD
Middle	12	12	12	TBD	TBD
High	12	12	12	TBD	TBD

THYROID HORMONE DETERMINATIONS

Serum from sacrificed animals will be analyzed to evaluate thyroid function. Commercially available (Radim-Techland, S.A. distributed by Wein Laboratories, Inc., Succasunna, NJ) I-125 labeled radio immune assay (RIA) kits will be used to measure thyroglobulin (Tg) and reverse triiodothyronine (rT_3). Species-specific RIA kits (Amersham, Inc. or equivalent) will be used for thyroid stimulating hormone (TSH). Thyronine uptake (TU) and tetraiodothyronine (T_4) levels will be determined using the DuPont ACA IV analyzer, and the free thyroxine index (FTI) will be calculated from these values to estimate the triiodothyronine (T_3) level. T_3 will be analyzed by RIA (Roche, Inc. or Amersham, Inc.).

USE OF EXPERIMENTAL DATA

Data obtained from this study will be used to statistically derive the threshold level for AP effects on the thyroid, the target organ for toxicity. The threshold level will be used as the LOAEL for determination of the RfD for ammonium perchlorate using standard USEPA methodology.

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